Copy for the Elected Office (EO/US)

PCT/GB97/02284

PATENT COOPERATION TREATY

9/242843

PCT

From the INTERNATIONAL BUREAU

To:

SKELTON, S., R.
D/IPR
Formalities Section (Procurement Executive)
Poplar 2, MOD Abbey Wood #19
P.O. Box 702
Bristol BS12 7DU
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
09 June 1999 (09.06.99)

Applicant's or agent's file reference
1G/P0088/WOD

IMPORTANT NOTIFICATION

International application No.
PCT/GB97/02284

International filing date (day/month/year)
27 August 1997 (27.08.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
THE MINISTER OF AGRICULTURE
FISHERIES & FOOD
Whitehall Place
London SW1A 2HH
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
THE MINISTER OF AGRICULTURE
FISHERIES & FOOD IN HER BRITANNIC
MAJESTY'S GOVERNMENT OF THE UNITED
KINGDOM OF GREAT BRITAIN &
NORTHERN IRELAND
Whitehall Place
London SW1A 2HH
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:
The name of the applicant has been corrected to read as stated in box 2

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
Catherine Massetti
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 March 1998 (25.03.98)	
International application No. PCT/GB97/02284	**Applicant's or agent's file reference** 1G/P0088/WOD
International filing date (day/month/year) 27 August 1997 (27.08.97)	**Priority date** (day/month/year) 29 August 1996 (29.08.96)
Applicant JARRETT, Paul et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   07 March 1998 (07.03.98)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Carlos Roy
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
D/IPR (DERA) Formalities
Poplar 2
Attn. SKELTON SR et al.
MOD(PE) Abbey Wood 19
PO Box 702
Bristol BS12 7DU
UNITED KINGDOM

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference IG/P0088/WOD	Date of mailing *(day/month/year)* 14/01/1998 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/GB 97/ 02284	International filing date *(day/month/year)* 27/08/1997
Applicant THE MIN. OF AGRICULTURE FISHERIES & FOOD et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Michael Davidson

Form PCT/ISA/220 (January 1994)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
IG/P0088/WOD		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 97/02284	27/08/1997	29/08/1996
Applicant		
THE MIN. OF AGRICULTURE FISHERIES & FOOD et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] *The international application contains disclosure of a* **nucleotide and/or amino acid sequence listing** *and the* international search was carried out on the basis of the sequence listing

   [X] filed with the international application.

   [ ] furnished by the applicant separately from the international application,

   [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] *the text is approved as submitted by the applicant*

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/02 A01N63/00 C12N1/20 C07K14/24 //(A01N63/02, 63:02,63:00),(A01N63/00,63:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00647 A (COMMW SCIENT IND RES ORG ;SMIGIELSKI ADAM JOSEPH (AU); AKHURST RAY) 5 January 1995 cited in the application	1,5,11, 13, 18-21, 24-26, 29,30,32
Y	see page 1, line 3 - line 29; claims 10-13	3,4, 6-10,12, 14,27, 28,31

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	14/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muellners, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 118, no. 1, 4 January 1993 Columbus, Ohio, US; abstract no. 3550, YAMANAKA, SATOSHI ET AL: "Biochemical and physiological characteristics of Xenorhabdus species, symbiotically associated with entomopathogenic nematodes including Steinernema kushidai and their pathogenicity against Spodoptera litura (Lepidoptera: Noctuidae)" XP002048914 see abstract & ARCH. MICROBIOL. (1992), 158(6), 387-93 CODEN: AMICCW;ISSN: 0302-8933, 1992, ---	3,6
Y	DATABASE DISSABS STN-International / UMI Company STN-AN 96:33246, DISSABS order no. AAI9608671 , 1995 DAVID JOSEPH BOWEN : "Characterization of a High Molecular Weight Insecticidal Protein Complex Produced by the Entomopathogenic Bacterium Photorhabdus luminescens (Nematodes, Biological Control)" XP002048915 see abstract & DISSERTATION ABSTRACTS JOURNAL INTERNATIONAL , vol. 57, no. 1B, 1995, page 93 ---	4,12,14
Y	EP 0 238 441 A (CIBA GEIGY AG) 23 September 1987 see page 1 - page 2 see page 4, paragraph 3 - page 5, paragraph 2; claims 10,12,22,36,37 ---	7-10,27, 28,31
X	WO 84 01775 A (COMMW SCIENT IND RES ORG ;BIOTECH AUSTRALIA PTY LTD (AU)) 10 May 1984 cited in the application see page 1 - page 3, line 10 see page 4, line 24 - line 28 see page 4, line 36 - page 5, line 3 see page 14, line 17 - line 29 see claims 26,27 ---	1,4,5, 11,13

1

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	H.MATSUI ET AL. : "Nucleotide sequences of genes encoding 32 KDa and 70 kDa polypeptides in mba region of the virulence plasmid, pKDSC50, of Salmonella choleraesuis " NUCLEIC ACIDS RESEARCH , vol. 18, no. 8, 1990, pages 2181-2, XP002050055 see the whole document ---	21-25
X	F.BINDER ET AL.: "Cyclodextrin-glycosyltransferase from Klebsiella pneumoniae M5a1: cloning nucleotide sequence and expression" GENE, vol. 47, 1986, pages 269-77, XP002050056 see page 269, the summary see page 270, right-hand column, last paragraph - page 271, right-hand column, paragraph 1 see fig. 3 bp 2641-2809 ---	21-25
P,X	US 5 616 318 A (DUDNEY RALPH A) 1 April 1997 see column 1, line 65 - column 2, line 52 see column 5, line 3 - line 4 ---	1,4-6, 11,13
T	WO 97 17432 A (WISCONSIN ALUMNI RES FOUND) 15 May 1997 see page 2, line 31 - page 3, line 23 see page 5, line 1 - line 16 see page 8, line 23 - line 33 see page 9, line 41 - page 11, line 14 see page 17, line 1 - line 21 -----	1-32

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/02284

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9500647 A	05-01-95	AU 675335 B	30-01-97
		AU 6991694 A	17-01-95
		EP 0705340 A	10-04-96
		JP 9500264 T	14-01-97
EP 0238441 A	23-09-87	GB 2188049 A	23-09-87
		AU 608508 B	11-04-91
		AU 6999287 A	17-09-87
		BG 46006 A	15-09-89
		BG 46752 A	15-02-90
		BR 8701162 A	12-01-88
		DE 3788077 D	16-12-93
		DK 128687 A	16-09-87
		EG 18869 A	28-02-94
		ES 2059404 T	16-11-94
		IE 59456 B	23-02-94
		JP 62224295 A	02-10-87
WO 8401775 A	10-05-84	AU 558287 B	22-01-87
		CA 1214130 A	18-11-86
		EP 0126092 A	28-11-84
		US 4672130 A	09-06-87
US 5616318 A	01-04-97	NONE	
WO 9717432 A	15-05-97	AU 1050997 A	29-05-97
		CA 2209659 A	15-05-97
		EP 0797659 A	01-10-97

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SMK/BP5703830		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/GB 97/ 02284	27/08/1997	29/08/1996
International Patent Classification (IPC) or national classification and IPC		
A01N63/02		
Applicant		
THE MIN. OF AGRICULTURE FISHERIES & FOOD et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 14 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/03/1998	03. 12. 1998
Name and mailing address of the IPEA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Netherlands Tel.: (+31-70) 340-2040, Tx. 31 651 epo nl Fax: (+31-70) 340-3016	Mollner W. Telephone No. 3289

INTERNATIONAL PRELIMINARY EXAMINATION REPORT	International application No. PCT/GB97/02284

I. Basis of the report

1. This report has been drawn up on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*

☐ the international application as originally filed

☒	the description, pages	1, 3-25	, as originally filed	
	pages		, filed with the demand	
	pages	2	, filed with the letter of	02.10.98
☒	the claims, Nos.	9 - 27	, as originally filed	
	Nos.		, as amended under Article 19	
	Nos.		, filed with the demand	
	Nos.	1 - 8, 28 - 32	, filed with the letter of	02.10.98
☒	the drawings, sheets / fig.	1/13 - 13/13	, as originally filed	
	sheets / fig.		, filed with the demand	
	sheets / fig.		, filed with the letter of	

2. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.

☐ the drawings, sheets / fig.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2 (c)).

4. Additional observations, if necessary:

III. **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ *the entire international application,*

☒ claims Nos. 25 - 29 (all partially)

because:

☒ the said international application, or the said claims relate to the following Nos. subject matter which does not require an international preliminary examination *(specify)*:

The European Patent Office as the International Preliminary Examining Authority is not required to carry out an international preliminary examination in respect of an international application on subjects related to plant varieties (Article 34(4)(a)(I), Rule 67.1 ii)). In their present wording these claims relate partially (claims 25-28) or entirely (claim 29) to plant varieties.
This assessment is not based on the assumption that they cover plants which have been treated in accordance with the invention, and which could embrace known plant varieties which have been so treated, but on the fact that a genetic modification according to claim 24 itself makes such transformed plants plant varieties.
However disregarding the form and kind of these claims and taking into account their entire content it is considered that the claimed subject-matter also provides technical features, which could perhaps be made the subject-matter of amended claims not related to excluded subject-matter, e.g. a claim directed to transformed plant cells, which can be propagated and manipulated in a laboratory would not be considered to fall under the definition of a plant or a plant variety. Therefore an international preliminary examination was carried out for these aspects of the subject-matter of claims 25-29 (PCT Preliminary Examination Guidelines IV.-2.6).

☐ the description, claims or drawings *(indicate particular elements below)* or said claims are so unclear that no meaningful opinion could be formed *(specify)*: Nos.

☐ the claims, or said claims are so inadequately supported by the description no meaningful opinion could be formed. Nos.

☐ no international search report has been stablished for said claims Nos.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Claims	2, 7 - 10, 12 and 15 - 32	YES
	Claims	1, 3 - 6, 11, 13 and 14	NO
Inventive Step	Claims	2, 15 - 17, 22 and 23	YES
	Claims	1, 3 - 14, 18- 21 and 24 - 32	NO
Industrial Applicability	Claims	1 - 32	YES
	Claims		NO

2. Citations and Explanations

The following documents (D1-D7, and D10) have been considered for the purposes of this report. Documents D1-D7 were cited in the international search report, document D10 was cited by the applicant in his response on the first written opinion of the International Preliminary Examination Authority:

D1 WO 95/00647 A
D2 Chem. Abs. 118(1), 3550 (1993)
D3 Diss. Abs., order no. AAI9608671 (1995) [STN-accession no. 96:33246]
D4 EP 0 238 441 A
D5 WO 84/01775 A
D6 Nucleic Acid Research 18(8), 2181-2 (1990)
D7 Gene 47, 269-77 (1986)
D10 International Journal of Systematic Bacteriology 43, 249-55 (1993)

Novelty:

1.1 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1, 3 - 6, 11, 13 and 14, insofar as clear, is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

1.1.1 Document D1 discloses proteinaceous toxins obtainable from a strain of the bacterium *Xenorhabdus nematophilus* which is able to kill insects, e.g. *Galleria mellonella* (greater wax moth, *Lepidoptera*) corresponding polynucleotides and recombinant organism including transformed plants (see D1, page 1, lines 3-29 and claims 10-13).

The bacterium is characterised as being toxic without the aid of an insect pathogenic nematode it is normally symbiotically associated with (see page 1, lines 16-19). Thereby the document discloses that the bacterium does not require a nematode that punctures through the insect surface and releases (i.e. effectively injects) it into the insect. This cannot be interpreted as referring to the obvious experimental route commonly used when studying the insecticidal properties of such bacteria to mimic what the nematode does by injecting bacteria directly in to the insect's body in the absence of nematodes, as it is also done in document D1 when screening for toxin producing clones (see page 4, paragraph 2 and page 5, paragraphs 2-3). Instead is has to be interpreted as indicating that the protein toxin has toxic activity when administered orally. This is confirmed by the fact that D1 - much the same as the present application - envisages the cloning of the sequence into host organisms like nuclear polyhedrosis virus and plants which can only be effective if the protein is toxic when ingested (see D1, page 8, lines 9-27 and claims 11and 12). Again much the same as the present application it does not exemplify the insertion of corresponding nucleotide sequence into a host organism but this can be done by the skilled person routinely using general methodology (see the present application, page 11, lines 34-38).

The following has also to be pointed out:
The description of the present application when referring to D1 states that there is no disclosure of the use of the toxic protein disclosed in D1 as an oral insecticide (see page 2, lines 23-27). The claims of the present application are not restricted to use claims but instead claims are directed i.a. to: compositions adapted for oral administration, comprising a proteinaceous toxin, and having toxic activity when administered orally, thus without restriction to the exemplified toxin (independent claim 1) and methods for killing or controlling insects comprising administering to a pest or the environment such a composition (independent claim 13).
The method of D1 comprising applying to an area a recombinant organism optionally in admixture with an acceptable agricultural carrier (see D1 originally filed claim 13 and page 3, lines 15-19) is indistinguishable from a method according to claim 13 of the present application. The compositions used in this method even if they were not intended for oral ingestion by the insect, like for instance recombinant plants by lepidopteran pests definitely are, would probably be orally ingestible by it and are thus indistinguishable from composition adapted for oral administration according to claim 1 of the present application

Therefore the disclosure of document D1 is considered to be novelty destroying for the subject-matter of claims 1, 5, 11, 13 and 14.

1.1.2 Document D2 discloses that the supernatant of a culture of strain ATCC 19061 of *Xenorhabdus nematophilus* was pathogenic for *Spodoptera litura* upon injection and was

inactivated by heat. Cells and supernatants of another *Xenorhabdus nematophilus* strain were not pathogenic upon injection, while still other strains showed pathogenicity for whole cell injections.
It has again to be emphasised that the claims are not restricted to uses but claim compositions per se. Document D2 discloses the supernatant of *Xenorhabdus nematophilus* strain ATCC 19061 which is one of the preferred strains of the present application (see claim 6). Example 2 of the present application demonstrates that its supernatant is an oral insecticide and consequently a composition within the scope of claim 1.
Consequently document D2, since it discloses this supernatant and thereby implicitly also the the cells, is novelty destroying for the subject-matter of claims 1, 3 - 6 and 11.

1.2 The subject-matter of claims 2, 7-10, 12, 15-32, insofar as clear, satisfies the criterion set forth in Article 33(2) PCT because it is new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

1.2.1 Document D3 discloses that *Photorhabdus luminescens*, which is closely related to the genus Xenorhabdus and was formerly classified as *Xenorhabdus luminescens*, secretes a toxin which is lethal upon injection into or feeding to larvae of several orders of insects including *Lepidoptera* and is inactivated by heating.
Document D4 describes a DNA fragment and an insecticidal protein from a specific *Bacillus thuringiensis* strain, corresponding recombinant microorganisms and processes for controlling lepidopteran pests (see D3, claims 1, 4, 22, 36 and 37). It also proposes fusion proteins comprising a second toxic protein, like for instance a second insecticidal protein, but does not specify the second protein any further (see claims 10 and 12).
Document D5 discloses antibiotics and pesticidal low molecular weight compounds from *Xenorhabdus sp.* including *Xenorhabdus nematophilus* and corresponding formulations and pesticidal methods (see D5, page 1-page 3, line 10; page 4, lines 24-28; page 4,line 36-page 5, line 3; page 14, lines 17-29 and claims 26 and 27).

Thus the prior art of D1 to D5 does not disclose: compositions comprising material encoded by the sequence of figure 2 (claim 2), or beside material from *Xenorhabdus* a further pesticidal material not obtainable from *Xenorhabdus* (claim 7 and 8-10 and 12), Xenorhabdus strains NCIMB 40886 and NCIMB 40887 (claims 15 and 16), a pesticidal agent comprising a protein encoded by DNA including sequence no.1 (claim 17), a proteinaceous pesticidal agent obtainable from culture of *Xenorhabdus nematophilus* acting synergistically with *Bacillus thuringiensis* cells (claims 18 -20), a recombinant DNA encoding such pesticidal agents (claims 21-23) a corresponding expression vector (claim 24), a host organism transformed with such a

expression vector and/or comprising a nucleotide sequence encoding for a fusion protein comprising a portion obtainable from Xenorhabdus nematophilus (claims 25-30), a corresponding fusion protein (claim 31) or composition comprising such agents (claim 32).

1.2.2 In particular with regard to claims 2, 17, 22 and 23 it is remarked that the protein toxin and the corresponding encoding DNA of D1 does not appear to be related to the protein toxin encoded by sequence no. 1 of figure 2 and the sequence itself respectively.

Further Document D6 discloses genes on the virulence plasmid of *Salmonella choleraesuis* (*Enterobacteriaceae* like *Xenorhabdus*) which in the region of ca. 1250-2050 shows a 774 bp overlap with the region 13180-14000 of sequence no. 1.
In document D7 a sequence from *Klebsiella pneumoniae* (*Enterobacteriaceae*) 2642 to the end, downstream of the region apparently encoding for cyclodextrin-glycosyltransferase, shows in 139 bp a 74% identity with 30900-30760 of sequence no. 1.
These sequences as a whole are not fragments or variants according to the definition of the description (see page 3) of the sequence of figure 2 nor do the hybridise with it under stringent conditions as defined in the description (see page 7), but they comprise fragments which are 55% and 74% respectively homologue to fragments of sequence 1. However there is no indication that these overlapping regions (or any other region of the sequences disclosed of D6 and D7) encode for an insecticidal toxin (claim 22) or a pesticidal material in general (claim 23).

Inventive step:

1. The subject-matter of claims 1, 3 - 6, 11, 13 and 14 in as far as it covers compositions and methods known from the prior art (see above) can for this reason not involve an inventive step.
The present application does also not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1, 3-14, 18-21 and 24-32, insofar as new and clear, does not involve an inventive step either (Rule 65(1)(2) PCT).

The problem underlying the present application can be regarded as the provision of pesticidal material from bacteria that are effective when taken orally by the target pest and corresponding methods (see page 1, lines 10-21).
According to the claims (see in particular claims 1 and 18) the solution is the provision of pesticidal material obtainable from *Xenorhabdus* species and having oral pesticidal activity
The closest prior art is disclosed in document D3 (see above under point 1.2.1).

1.1. If the solution as defined above meets the requirements of Articles 5 and 6 PCT in conjunction with Rules 5.1(a) (iii) and Rule 6.3 (but see below under point VIII) it would follow

that obtaining such material does in principal not require inventive skill once *Xenorhabdus* species have been identified as potential source.
From document D3 it is known that another bacterium symbiotically associated with insect pathogenic nematodes, namely *Photorhabdus luminescens*, secretes a proteinaceous toxin that is toxic on feeding to insects including *Lepidoptera* and thus already provides a solution to the problem specified above.
This species is closely related to *Xenorhabdus* species and has actually only recently been removed from the genus *Xenorhabdus* (see the present application, page 1, lines 30-33, and for more details D10 and the later published document WO 97/17432 A (=D9), page 5, lines 1-16, this document is also discussed below under point VI).

The following has to be pointed out:
Photorhabdus luminescens is in its taxonomically relevant properties so different from other species of the genus *Xenorhabdus* under which it was (probably due to the for taxonomic purposes rather irrelevant fact that it too was isolated from nematodes) formerly classified, that it was concluded that it is phylogenetically not so closely related to justify grouping it under the same genus. Therefore a now generally accepted taxonomic revision was proposed allocating it into a new genus *Photorhabdus* (see document D10)
However with regard to properties relevant in the technical field of the present application, i.e. controlling insect, *Photorhabdus luminescens* shares decisive characteristics with species of *Xenorhabdus* in general and *X. nematophilus* in particular. They are both symbiotically associated with insect pathogenic nematodes which actively seek insect hosts, puncture them, and inject the toxin producing bacterium into the insect's haemocoel.
Thus, regardless whether they are phylogenetically totally distinct and the taxonomic revision is universally accepted, the skilled person working in the technical field of the application would have considered them for his purposes as closely related.
This is confirmed by the applicant himself as such a skilled person when writing in the description of his application: "In addition, one extracellular insecticidal toxin from *Photorhabdus luminescens* has been isolated (this species was recently removed from the genus *Xenorhabdus*, and is **closely related** to the species therein). This toxin is not effective when ingested ..." [emphasis added]. Thereby apparently wanting to imply that if a toxin of *Photorhabdus luminescens* is not effective when ingested the skilled person would expect toxins from *Xenorhabdus ssp.* not to be effective either because the two species are closely related.
Consequently if on the other side a toxin of *Photorhabdus luminescens* was found to be toxic upon ingestion, as is the case (see D3), the skilled person would then expect that searching for such orally active toxins *expressed by Xenorhabdus ssp.* is likely to be successful too.
Even more so since it was known from D1 that *Xenorhabdus nematophilus* is a source for

proteinaceous toxins exhibiting insecticidal activity without the aid of an insect pathogenic nematode. It was therefore obvious for the skilled person that this species was a suitable source for pesticidal material solving the underlying problem.
Thus it has to be concluded that insofar as the subject-matter of claims 1, 3-14, 18-21 and 24-32 is not disclosed in documents D1-D6, or - in particular with regard to subject-matter of claims 1, 3 - 6, 11, 13 and 14 - would be considered to be new despite the arguments presented above, it does not involve an inventive step.

1.1.1. In particular with regard to the subject-matter of claims 7-10, 12, 18-21 and 24-32 the additional features specified in these claims are features the skilled person would consider to introduce in the light of the teachings of the prior art.
The skilled person would consider orally active toxins obtainable from *Xenorhabdus* species to be suitable second proteins in fusion proteins as they were proposed in D4, i.e. fusion proteins comprising also an insecticidal protein from *Bacillus thuringiensis* (claims 7-10, 27, 28 and 31). From the fact that the toxin is like the toxin of D3 lethal upon feeding it is obvious to include into the formulation an item of insect diet (claim 12).
Especially with regard to claim 18, 27 and 28 it is remarked that the applicant has demonstrated a synergistic effect only for cells and supernatants of three strains of *Xenorhabdus nematophilus* with *Bacillus thuringiensis* and that he only proposes fusion proteins without exemplifying one. If the subject-matter of those claims and claims depending on them in as far as it goes beyond what was actually exemplified are considered to be sufficiently disclosed to enable a skilled person to realise them without an undue amount of experimentation and/or exercising inventive skill (Article 5 PCT, see also below under point VIII) it would follow that it does not involve an inventive step, because a (synergistically) enhanced effect of the known or obvious proteinaceous toxins comprised in compositions according to claim 18 is obviously desirable. Thus the skilled person would isolate the pesticidal agents and prepare the host organisms, provided it does not require an undue amount of experimentation and/or exercising inventive skill.

1.2 From the above it would follow that also the subject-matter of claims 2, 15-17, 22 and 23 does not involve an inventive step, because these specific solution would merely be representative examples of the results achieved by applying routine experimentation once, based on obvious considerations, *Xenorhabdus* was chosen as the suitable source for the desired pesticidal agents.

However it has rather to be assumed that the definition of the solution by the result to be achieved, i.e. that it solves the problem, does not meet the requirements of Articles 5 and 6 PCT in conjunction with Rules 5.1(a) (iii) and Rule 6.3. Because, although it is obvious that

Xenorhabdus is a likely source of suitable toxins and that therefore *Xenorhabdus* strains showing the desired properties will probably exist, these specific strains will nevertheless be so rare that actually finding one and identifying its proteinaceous toxin and the DNA encoding it has to be considered to require inventive activity.
Under this provision the subject-matter of claims 2, 15-17, 22 and 23 (and consequently those parts of the subject-matter of the remaining claims involving the additional features of these claims) would meet the requirements of Article 33(2)and (3) PCT in conjunction with (Rule 64(1)-(3) PCT) and (Rule 65(1)(2) (PCT) respectively.

Industrial Applicability

The subject-matter of claims 1-32 is considered to be industrially applicable; the claims therefore satisfy the criterion set forth in Article 33(4) PCT.

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application no. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
US 5 616 318 A	01/04/1997	09/06/1995	
WO 97/17432 A	15/09/1997	06/11/1996	

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

1. Document US 5 616 318 A (=D8) discloses the use of liquid cultures of certain *Xenorhabdus nematophilus* strains, including strain ATCC 19061, against fire ants (see column 1, line 65- column 2, line 52 and column 5, lines 3 and 4). It states (see column 2, lines 3-8) that it was believed that *Xenorhabdus* was not pathogenic for insects when ingested, thereby implying that it considers the specific strains to be pathogenic when ingested and the proposed method to be based on oral pesticidal activity.
The document was published after the priority date claimed for the present application but before the filing date of the present application with the International Bureau.
The priority documents pertaining to the present application were not available at the time of establishing of this report. Hence, it is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the document could become relevant to assess whether the claims satisfy the criteria set forth in Article 33, it would for instance be novelty destroying for claims 1, 4-6, 11 and 13 of the present application, for reasons analogous to those given above with regard to document D2 (see point V.,novelty, 1.1.2).

2. Document WO 97/17432 A (=D9) solves the same problem as the present application. It proposes *Photorhabdus luminescens*, which is closely related to the genus *Xenorhabdus* (see also above under point V), as the source for toxins having oral pesticidal activity against e.g. *Lepidoptera*. It also proposes host organisms including plants transformed with the DNA

encoding for that protein or for a fusion protein comprising this toxin and a toxin from *Bacillus thuringiensis* (see D8, page 2, line 31 - page 3, line 23; page 5, lines 1-16; page 8, lines 23-33; page 9, line 41-page 11, line 14; page 17, lines 1-21 and claims 1,2,10 and 21).

The document was also published after the priority date claimed for the present application but before the filing date of the present application with the International Bureau.

Since the document is directed to toxins from a *Photorhabdus* species not a *Xenorhabdus* species it does in any case not destroy the novelty of the subject-matter claimed in the present application.

However, if it later turns out that the present application does not enjoy priority rights from the filing date of the priority document, the document would become relevant to assess whether the claimed subject-matter involves an inventive step (Article 33(3) PCT in conjunction with Rule 65(1)(2) PCT). The document would become the closest prior art and analogous arguments based above under point V on document D3 could be based on it.

VII. Certain defects in the internati nal application

The following defects in the form or contents of the international application have been noted:

To meet the requirements of Rule 5.1(a)(ii) PCT, documents D2-D4 should also have been identified in the description and the relevant background art disclosed therein should have been briefly discussed.

VIII. C rtain observati ns on th internati nal applicati n

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. Claim 18 and by direct or indirect reference to it also claims 19-21 and 24-32 attempt to define the subject-matter in terms of the result to be achieved.
Such a definition of a feature by the result to be achieved, in particular if it amounts to claiming the successful solution of the underlying technical problem, is only acceptable if it can only be defined in such terms or cannot otherwise be defined more precisely without unduly restricting the scope of the claims and if the result is can be achieved by the skilled person without an undue amount of experimentation and/or requirement of inventive skill. This would imply that identifying suitable *Xenorhabdus nematophilus* strains and further identifying and isolating the desired pesticidal agents and/or the DNA encoding them from these strains does not require inventive skill and that consequently the entire claimed subject-matter does not involve an inventive step (see above under point V).
If on the other hand identifying the features of claims 2, 15-17, 22 and 23, which now due to the teaching of the present application allow the skilled person to achieve the desired result without undue experimentation or requirement of inventive skill, required the inventive skill of the applicant other solutions to the underlying problem covered by claims 18-21 and 24-32 than those characterised by said features are not sufficiently disclosed. These claims would therefore not meet the requirements of Articles 5 and 6 PCT in conjunction with Rules 5.1(a)(iii) and Rule 6.3.

2. In claim 6 it should read "strain" instead of "species"

3. Claim 14 should depend on claim13 rather than claim 12.

4. The sequence should have been consistently defined either as "sequence of Figure 2" or as "SEQ ID No. 1" or both throughout the claims (claims 2, 17 and 22).

5. There is no certificate or an equivalent proof regarding the deposition of strains NCIMB 40886 and NCIMB 40887 in accordance with the requirements of Rule 13bis PCT on file. It is therefore not possible to decide definitely whether the subject-matter of the claims meet the requirement of Article 5 PCT in conjunction with Rule 13bis PCT.

CLAIMS:

1. An insecticidal composition which:
(i) is adapted for oral administration to an insect,
(ii) comprises a proteinaceous pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these,
having in each case toxic activity when administered orally.

2. A composition according to claim 1 wherein the said pesticidal material comprises material encoded by the nucleotide sequence of Figure 2 or variant or fragment thereof, or a sequence which hybridises with said sequence.

3. A composition according to claim 1 or claim 2 which comprises cells of *Xenorhabdus*.

4. A composition as claimed in any one of the preceding claims which comprises supernatant taken from cultures of cells of *Xenorhabdus* species.

5. A composition according to any one of the preceding claims wherein the *Xenorhabdus* species is *Xenorhabdus nematophilus*.

6. A composition according to any one of claims 1 to 4 wherein the *Xenorhabdus* species is ATCC 19061, NCIMB 40886 or NCIMB 40887.

7. A composition as claimed in any one of the preceding claims which comprises a further pesticidal material not obtainable from *Xenorhabdus*.

8. A composition according to claim 7 wherein the said further pesticidal material comprises a material obtainable from *B. thuringiensis*.

28. A host organism as claimed in claim 27 wherein the pesticidal toxicity enhancing materials comprise delta-endotoxin from B. thuringiensis.

29. A host organism as claimed in any one of claims 25 to 28 wherein the host is a plant.

30. A host organism as claimed in any one of claims 25 to 28 wherein the host is a virus pathogenic to insects.

31. A fusion protein as expressed by a host as claimed in claim 27.

32. A pesticidal composition comprising one or more agents as claimed in any one of claims 17 to 20.

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Typed Name of Person Mailing Paper or Fee

Tina M. Doyle
Signature of Person Mailing Paper or Fee

Re: International Application No. PCT/GB97/02284
International Filing Date: 27 August 1997
Priority Date: 29 August 1996
Applicants: Paul JARRETT et al.

Enclosures:

- Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under 35 USC 371
- Preliminary Amendment
- Copy of Article 34(2)(b) claim amendments (substitute pages 26 and 29)
- Preliminary Amendment
- Check for $1492.00

REPLACED BY ARTICLE 34

Also known are certain low-molecular weight heterocyclic compounds from *P.luminescens* and *X.nematophilus* which have antibiotic properties when applied intravenously or topically (see Rhodes, S.H. et al., PCT WO 84/01775).

Unfortunately none of these prior art materials have the ideal pesticide characteristics discussed above, and in particular, they do not have toxic activity when administered orally.

The present invention provides pesticidal agents and compositions from *Xenorhabdus* species, organisms which produce such compounds and compositions, and methods which employ these agents, compositions and organisms, that alleviate some of the problems with the prior art.

According to one aspect of the present invention there is disclosed a method of killing or controlling insect pests comprising administering cells from *Xenorhabdus* species or pesticidal materials derived or obtainable therefrom, orally to the pests.

A PCT application of CSIRO published as WO 95/00647 discloses an apparently toxic protein from *Xenorhabdus nematophilus*; however no details of the protein's toxicity are given, and certainly there is no disclosure of its use as an oral insecticide.

Thus the invention provides an insecticidal composition adapted for oral administration to an insect, which composition comprises a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

The composition may in fact comprise cells of *Xenorhabdus* or alternatively supernatant taken from cultures of cells of *Xenorhabdus* species. However, the composition

CLAIMS

1. An insecticidal composition adapted for oral administration to an insect comprising a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

2. A composition according to claim 1 wherein the said pesticidal material comprises material encoded by the nucleotide sequence of Figure 2 or variant or fragment thereof, or a sequence which hybridises with said sequence.

3. A composition according to claim 1 or claim 2 which comprises cells of *Xenorhabdus*.

4. A composition as claimed in any one of the preceding claims which comprises supernatant taken from cultures of cells of *Xenorhabdus* species.

5. A composition according to any one of the preceding claims wherein the *Xenorhabdus* species is *Xenorhabdus nematophilus*.

6. A composition according to any one of claims 1 to 4 wherein the *Xenorhabdus* species is ATCC 19061, NCIMB 40886 or NCIMB 40887.

7. A composition as claimed in any one of the preceding claims which comprises a further pesticidal material not obtainable from *Xenorhabdus*.

8. A composition according to claim 7 wherein the said further pesticidal material comprises a material obtainable from *B. thuringiensis*.

28. A host organism as claimed in claim 27 wherein the pesticidal toxicity enhancing materials comprise delta-endotoxin from B. thuringiensis.

29. A host organism as claimed in any one of claims 25 to 289 wherein the host is a plant.

30. A host organism as claimed in any one of claims 25 to 28 wherein the host is a virus pathogenic to insects.

31. A fusion protein as expressed by a host as claimed in claim 27.

32. An pesticidal composition comprising one or more agents as claimed in any one of claims 17 to 20.

CORRECTED VERSION*

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(54) Title: PESTICIDAL AGENTS

(57) Abstract

A method for killing pests (e.g. insects) comprising administering material from *Xenorhabdus* species (e.g. *X. nematophilus*) such as cells or supernatants orally to the pests, either alone or in conjunction with *Bacillus thuringiensis* or pesticidal materials derived therefrom. Also disclosed is an isolated pesticidal agent (and compositions comprising the same) characterised in that it is obtainable from cultures of *X. nematophilus* or mutants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55 °C, is proteinaceous, acts synergistically with *B. thuringiensis* cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K. DNA encoding pesticidal activity is also disclosed.

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INTERNATIONAL SEARCH REPORT

rnational Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/02 A01N63/00 C12N1/20 C07K14/24 //(A01N63/02, 63:02,63:00),(A01N63/00,63:00)

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropnate, of the relevant passages	Relevant to claim No.
X	WO 95 00647 A (COMMW SCIENT IND RES ORG ;SMIGIELSKI ADAM JOSEPH (AU); AKHURST RAY) 5 January 1995 cited in the application	1,5,11, 13, 18-21, 24-26, 29,30,32
Y	see page 1, line 3 - line 29; claims 10-13	3,4, 6-10,12, 14,27, 28,31

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	14/01/1998

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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